

Amendments to the Specification:

Please replace the Title beginning at page 1, line 1 with the following rewritten Title:

~~The lactose operon of Lactobacillus delbrueckii and its use for controlling gene transcription and/or expression in bacterial cells~~

Expression Constructs Using the *Lactobacillus delbrueckii* subsp. *lactis* Lac Repressor Protein and its Lac Repressor Binding Site, Microorganisms and Methods Thereof

Please replace the paragraph beginning at page 15, line 1 with the following rewritten paragraph:

31 Competent cells and transformations of Lactococcus lactis MG 1363 (~~plasmid free derivative of NCDO 712; Gasson, M.J., NCDO (1983) 712) and other lactic streptococci after protoplast induced curing, J. Bacteriol. 154:1-9~~) were realized according to Holo et al. Appl. Environ. Microbiol. **55** (1989), 3119-3123. Cells were grown in GM17-broth containing 500 mM sucrose and 3% glycine to an OD₆₀₀ of 0.2 - 0.3, centrifuged and washed several times in a solution containing 500 mM sucrose and 10% glycerol. Competent cells were stored 100x concentrated at -80°C in the same solution until use.

Please replace the paragraph beginning at page 19, line 1 with the following rewritten paragraph:

Example 3

Cloning of the *L. delbrueckii* lac promoter in front of the chloramphenicol acetyltransferase gene in *Escherichia coli*

32 The lac promoters of *L. delbrueckii* LL44, N141 and N299 were tentatively cloned in front of an *E. coli* reporter gene, the chloramphenicol acetyltransferase (cat) gene. They were PCR amplified using the following primers

Eco RI
ATATTAGAATTCAGTGACTTAACTGG SEQ ID No. 12

Eco RI
ATATTAGAATTCAGTACTTTGACACCG SEQ ID No. 13

Eco RI
ATATTAGAATTCAAGAGGCTATATCGC SEQ ID No. 14

GGTTAATGCCGCCAAGT SEQ ID No. 15

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which contain an EcoRI site and primer 250 which is located in the lacS gene. The amplified fragments were digested with EcoRI and BspEI, which cleave in the primer sequence and 170 bp downstream the start codon of lacS. Restriction sites were filled with T₄ DNA polymerase, cloned in front of the promoterless cat gene of pKK232-8 digested with SmaI and transformed in E. coli. Chloramphenicol resistant clones were obtained for the three lactobacilli lac promoters, which indicates that the lac promoters of L. delbrueckii are active in E. coli. They were called pLL55 for N299, pLL57 for N141 and pLL58 for LL44 (Table 2) and all the constructs were verified by sequencing.

Please replace the paragraph beginning at page 21, line 17 with the following rewritten paragraph:

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The same results were found for the Gram-positive bacterium Staphylococcus aureus, where the lac promoter was functional in E. coli but the lacR gene not (~~Oskouian et al, J. Bacteriol. 169 (1990), 5459-5465~~). One possible explanation is that an additional factor, present only in Gram-positive bacteria, is necessary for proper repressor function.

Please replace the paragraph beginning at page 25, line 18 with the following rewritten paragraph:

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In the presence of the LL44 lacR gene, cloned in both orientations behind the gusA gene (pLL115, for and pLL116, rev), almost no GusA activity was detected in the presence of mannose. This result indicates that the so called "constitutive" promoter of L. delbrueckii subsp. bulgaricus is in fact repressed by the L. delbrueckii lac repressor. In addition, increasing concentrations of lactose induced increasing production of GusA activity. Nevertheless, the production even at 1% lactose with the repressor in the reverse orientation (pLL116) reached only half of that obtained in the absence of repressor. The N299 is not only regulated by the repressor, but more tightly than that of LL44 (Table V).

Please replace the paragraph beginning at page 27, line 20 with the following rewritten paragraph:

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At all concentrations of lactose tested (0.02 to 0.5%), the β -gal activity was in the same range, indicating that very low concentration of lactose is able to fully induce the expression of the lac operon. In the presence of lactose and glucose, strains were not subjected to catabolite repression and even a stimulation of β -gal activity was found for N62 (Table 3, above). The

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constitutive *L. delbrueckii* subsp. *bulgaricus* N299 showed the same β -gal activity with all sugars used even in the presence of glucose. Two mutants of *L. delbrueckii* subsp. *lactis*, LB10 and LZL102, were also analysed. LZL102, the spontaneous mutant of LL44, produces a truncated repressor and LB10 produces no peptide at all (Fig. 12). In both cases, strains were constitutive with equal amounts of β -gal produced with galactose, lactose or glucose.

Please replace the paragraph beginning at page 32, line 1 with the following rewritten paragraph:

Summary **ABSTRACT OF THE DISCLOSURE**

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~~The present invention relates to a~~ A DNA sequence suitable for the controlled transcription and/or expression of a variety of different genes in bacteria, preferably gram positive bacteria is provided. In particular, the ~~present invention pertains to a~~ DNA sequence ~~comprising~~ includes the promoter and the gene coding for the lac repressor of the lac operon of *Lactobacillus delbrueckii* with a DNA sequence coding for a gene product of interest being arranged inbetween.